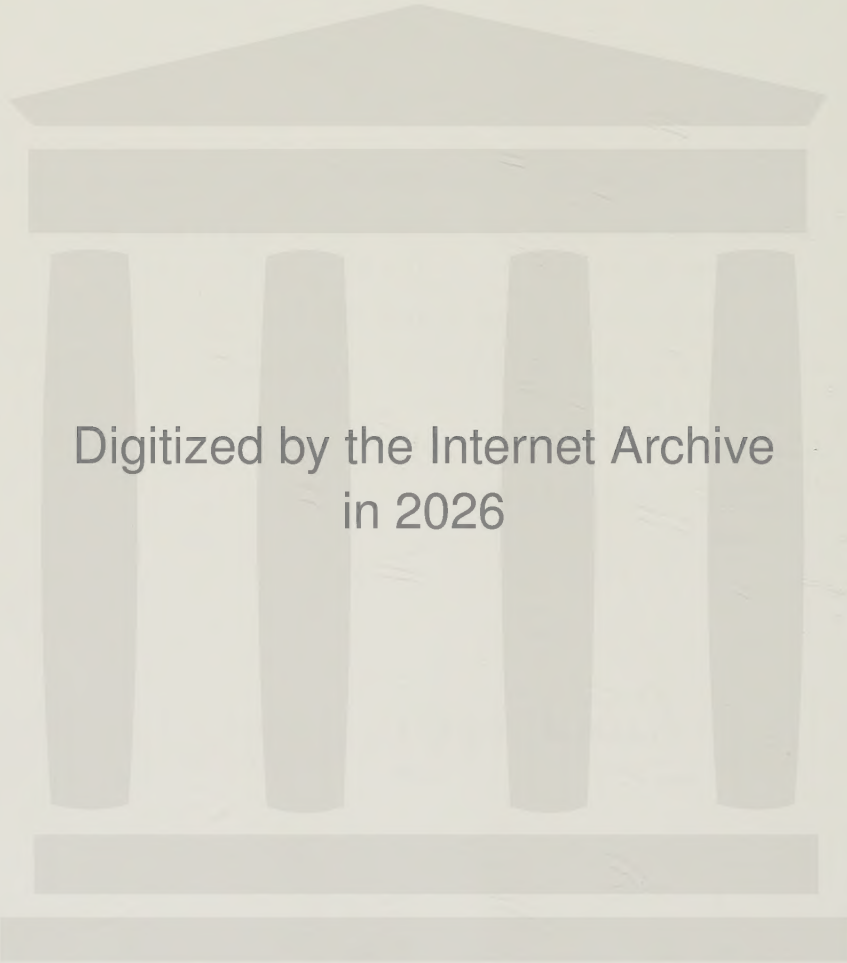


PENETRATION OF AMPICILLIN, DOXYCYCLINE
AND GENTAMICIN INTO INTERSTITIAL FLUID
IN RABBITS AND OF PENICILLIN V AND
PIVAMPICILLIN IN HUMANS MEASURED WITH
SUBCUTANEOUSLY IMPLANTED COTTON
THREADS

B. Hoffstedt & M. Walder

 Lund 1981



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Penetration of Ampicillin, Doxycycline and Gentamicin into Interstitial Fluid in Rabbits and of Penicillin V and Pivampicillin in Humans Measured with Subcutaneously Implanted Cotton Threads

Summary: An important prerequisite for therapeutic success in antibiotic treatment is that the drug reaches the focus of infection. Using subcutaneously implanted cotton threads we studied the penetration of five antibiotics into a non-inflammatory extravascular fluid in rabbits and human volunteers. The penetration of ampicillin in rabbits was 60% (calculated as the area under the tissue fluid concentration curve/the area under the serum concentration curve $\times 100\%$). It was 40% for doxycycline after a single dose and 65% in "steady state" and 60% for gentamicin. In humans, the penetration of oral penicillin V was 10% and of ampicillin 40%, both in steady state.

Zusammenfassung: Penetration von Ampicillin, Doxycyclin und Gentamicin in die Interstitialflüssigkeit bei Kaninchen und von Penicillin V und Pivampicillin bei Menschen, gemessen mit subkutan implantierten Baumwollbändern. Von großer Bedeutung in der Antibiotikatherapie ist die Frage, ob die Substanz den Herd der Infektion erreicht. In dieser Untersuchung haben wir die Penetration von fünf Antibiotika in eine nicht entzündliche extravaskuläre Flüssigkeit in Kaninchen und bei freiwilligen Versuchspersonen studiert. Die Flüssigkeit wurde mit subkutanen Bändern aus Baumwolle gesammelt. Die Penetration von Ampicillin in Kaninchen war 60% (berechnet als Fläche unter der Geweeflüssigkeitskonzentrationskurve/Fläche unter der Serumkonzentrationskurve $\times 100\%$), für Doxycyclin 40% nach einfacher Dosis und 65% im „steady state“ und für Gentamicin 60%. Bei Menschen war die Penetration von peroralem Penicillin V 10% und von Ampicillin 40%, gemessen im „steady state“.

Introduction

An important prerequisite for therapeutic success is that the antibiotic reaches the focus of infection. Many methods have been presented for collecting interstitial fluid (IF), e. g. subcutaneous chambers (1, 2, 3), skin windows (4), skin blisters (5, 6), diffusion chambers (7, 8, 9) and filter paper discs (10).

However, most of these methods have disadvantages and are difficult to use in patients. Furthermore, some of

them do not reflect the conditions of true non-inflammatory IF; instead, they tend to reflect the conditions of an abscess (11).

A novel method with subcutaneously implanted cotton threads has recently been described (12) and after further development it is easy to use in animals, patients and volunteers.

Materials and Methods

Three rabbits were used for each antibiotic. For penicillin V and pivampicillin investigations were carried out on two volunteers. At least one week passed before another antibiotic was administered.

We used an "umbilical tape" made of cotton thread, manufactured by Ethicon. It is an inert material with an absorption capacity of about 70% to 80% of its weight. The threads were manufactured in cut pieces 20 cm in length, attached to a nontraumatic needle and sterilized by Johnson & Co, Sweden. Six to seven samples could be obtained from each thread. After disinfecting the skin and applying local anaesthesia the threads were placed subcutaneously in the rabbits' back, and in the ventral surface of the humans' forearm (Figure 1). 1–2 cm of thread was inserted subcutaneously. Two pieces of thread were implanted in order to obtain paired samples from each sampling

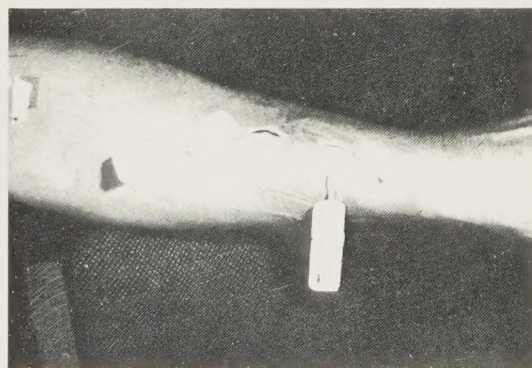


Figure 1: The forearm of a human volunteer with inserted subcutaneous cotton threads.

Received: 25 August 1980

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time. Blood contamination was no problem, neither in rabbits nor in humans. The blood content, measured with a spectrophotometer after eluting the thread content in sterile water, was always $< 5 \text{ g/l}$ in the analysed threads. However, most of the threads were not contaminated. The threads were implanted at least 30 min before the antibiotic was given. Serum samples and samples from the threads were taken simultaneously. The thread sample was gradually drawn through subcutaneously and then cut. The thread was then placed in a small tube and stored at -20°C together with the serum samples until they were assayed.

The average amount of fluid in the threads was 0.0057 g/cm corresponding to 74% of their weight.

Bacteriological methods: The antibiotic concentrations of the threads were determined by a microbiological assay. 1 cm of thread was cut into smaller pieces and spread out in an 8 mm well in an agar plate (Figure 2). 50 μl of buffer was added to each well to insure an even distribution of the antibiotic. The

agar used was DST 125 ml in 12×12 disposable plastic assay plates. The indicator organisms were *Bacillus cereus*, ATCC 11778, for doxycycline, *Micrococcus luteus*, ATCC 9341, for ampicillin and penicillin V, and *Staphylococcus epidermidis*, ATCC 5512, for gentamicin. Thread antibiotic standards were made by impregnating 1 cm of sterilized threads with different concentrations of antibiotic solutions. The standard curve for the threads was always parallel to the serum standard curve. The results obtained were influenced by high blood contamination in the threads which gave a false low value if the tested drug was bound strongly to serum protein. There was hardly any influence of blood contamination for drugs which were only slightly bound to serum protein.

The serum concentrations were measured with an ordinary agar well diffusion technique.

Results

We tested ampicillin, doxycycline and gentamicin in rabbits. The doses were 25 mg/kg, 1.5 mg/kg and 3 mg/kg respectively, given intravenously as short bolus injections. We studied the penetration of doxycycline into IF both in steady state, defined as the situation after three days of treatment, and after a single dose.

The half-life for ampicillin was 30 min both in serum and in IF, although the peak level in IF was delayed about 1 h. The penetration of ampicillin into tissue fluid (area under the tissue fluid curve/area under the serum level curve $\times 100\%$) was 60% (Figure 3). The area under the curve was calculated from the mean curve for each antibiotic in serum and interstitial fluid by the trapezoidal rule method. However, doxycycline had different values as could be expected from its pH, strong protein binding and long serum half-life since it is highly lipophilic. After a single dose of 1.5 mg/kg we found a half-life in serum and IF of about 3 h and the peak level in IF after 1 h. In

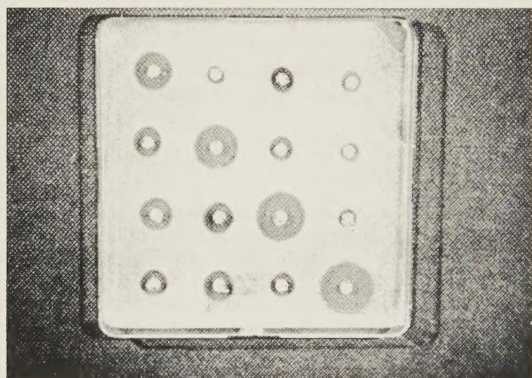


Figure 2: Assay plate for cotton threads.

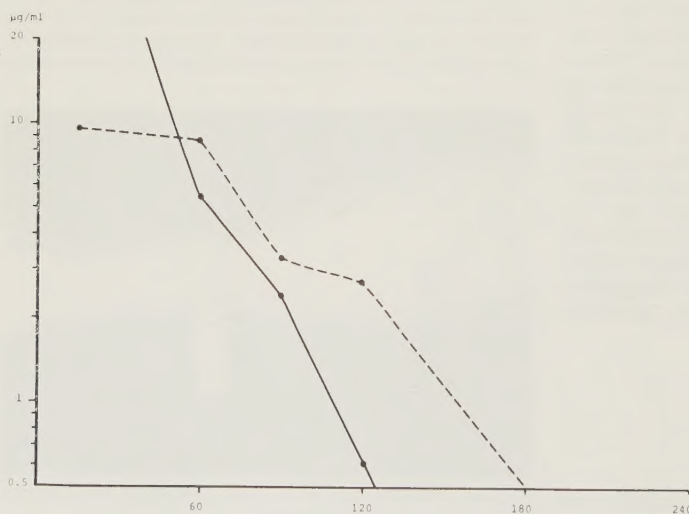


Figure 3: Serum and IF levels of ampicillin in rabbits. Dose: 25 mg/kg; Continuous line: Serum; Broken line: IF.



Figure 4: Serum and IF levels of doxycycline after a single dose in rabbits. Dose: 1.5 mg/kg; Continuous line: Serum; Broken line: IF.

"steady state" the half-life in serum was still about 3 h; it was prolonged in IF to about 4 h. The penetration after a single dose was 40% and in steady state 65% (Figures 4 and 5).

Gentamicin had a half-life of 2 h in serum and 2.5 h in IF. Penetration was 60% (Figure 6).

In humans, oral administration of penicillin V ($0.8 \text{ g} \times 3$) reached very low levels in IF in steady state. The half-life was 90 min both in IF and serum and penetration was only 10% (Figure 7).

Ampicillin, however, administered orally as pivampicillin ($0.35 \text{ g} \times 3$) had higher penetration, amounting to 40% in steady state. The half-life was 1 h in serum and 2.5 h in IF (Figure 8).

The accuracy for the serum assay was $\pm 25\%$ and for the IF assay $\pm 33\%$ within one standard deviation.

Chemical Analysis

The content of sodium and potassium in the fluid obtained in the cotton threads was, on the average, 140 meq/l and 4.0 meq/l respectively. The total protein content was 21 g/l in rabbits and 24 g/l in humans.

Discussion

Using subcutaneously implanted cotton threads is a convenient, non-traumatic method for obtaining physiological IF. The chemical analysis of the fluid obtained corresponds well with that of "true" IF (2, 13, 14). No side-effects were observed in rabbits or volunteers. The marks from implantation healed completely. In some rabbits the threads had been implanted for 48 h without any signs of infection.

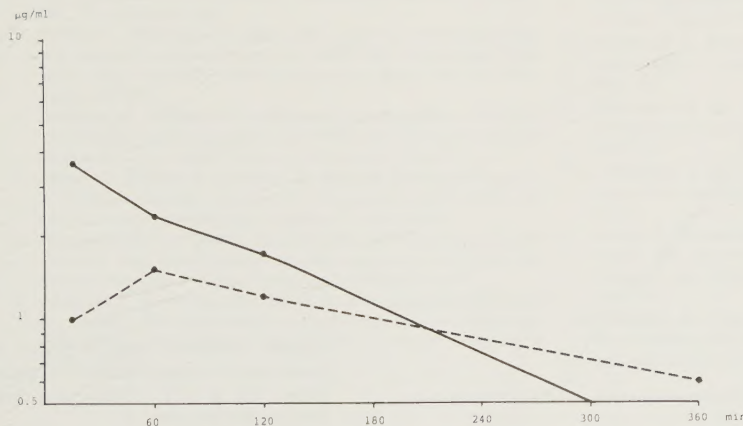


Figure 5: Serum and IF levels of doxycycline in steady state in rabbits. Dose: 1.5 mg/kg; Continuous line: Serum; Broken line: IF.

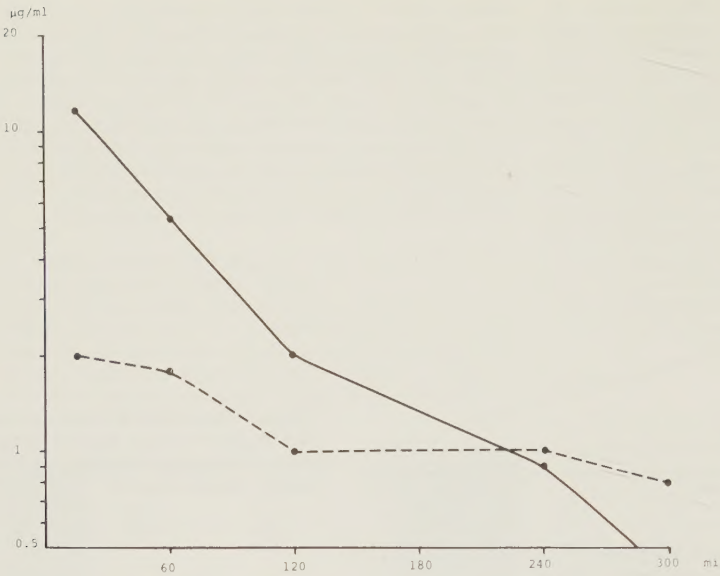


Figure 6: Serum and IF levels of gentamicin in rabbits. Dose: 3 mg/kg; Continuous line: Serum; Broken line: IF.

Our findings on the penetration properties of ampicillin into IF in rabbits are similar to observations already made (15). Notable is the cross-over point at one hour and the close similarity between serum and IF concentration curves, indicating good and free diffusion of ampicillin into IF. This is also verified by its high penetration rate of 60%. Doxycycline showed remarkably high penetration into IF in spite of its strong protein binding. Other authors found corresponding high levels in extravascular compartments

(16, 17, 18). These high levels could possibly be explained by the fact that only a small part of the doxycycline administered is bound to plasma proteins despite its high degree of binding and that doxycycline has a high affinity to different tissues. Gentamicin penetrated well into IF and gave a sustained level, corresponding well to other experiments (15). In volunteers pivampicillin penetrated much better into IF than penicillin V, although both substances disappeared rapidly from IF. The difference could be

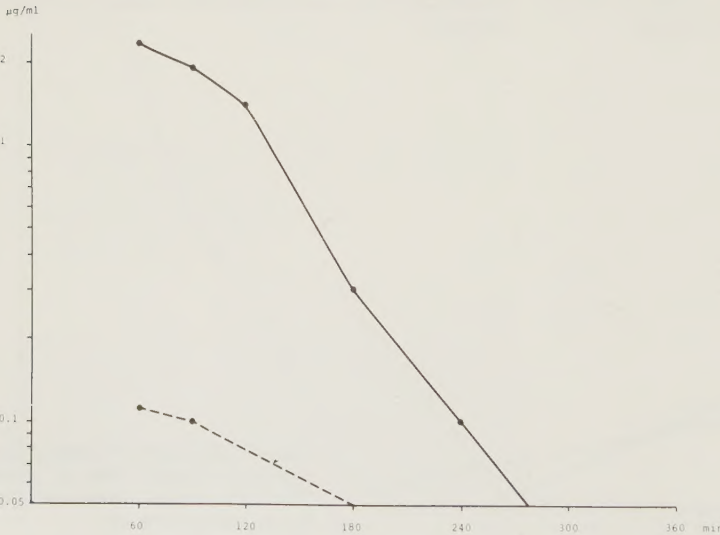


Figure 7: Serum and IF levels of penicillin V in steady state in humans. Dose: 0.8 g x 3; Continuous line: Serum; Broken line: IF.

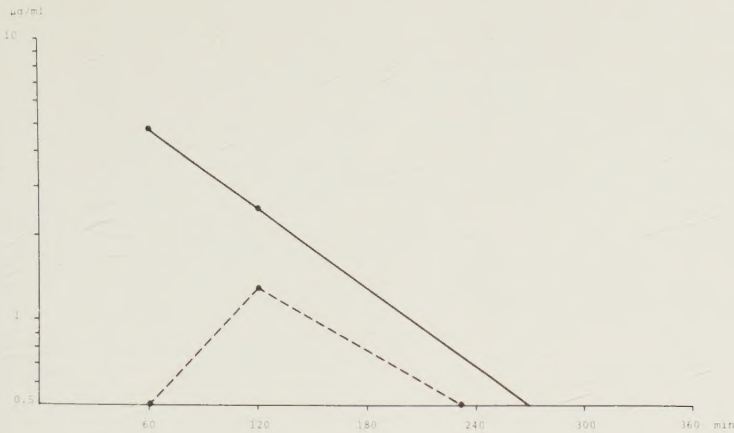


Figure 8: Serum and IF levels of ampicillin in steady state in humans. Dose: 0.35 g x 3; Continuous line: Serum; Broken line: IF.

explained fairly well by the difference in protein binding, and the results correspond well to other authors' findings (4).

A method for measuring antibiotic levels in non-inflammatory extravascular fluid can be of great clinical value since one important goal for antibiotic treatment is to avoid the spreading of infection. This is especially the case when treating surgical infections or when the drug is used for prophylaxis in surgery.

In summary, the method used to collect IF by subcutaneously implanted cotton threads is a convenient, reliable and simple method which could easily be used both in animals and in humans.

Acknowledgement

We thank Miss B. Larsson for skilful technical assistance.

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THE INFLUENCE OF SERUM PROTEIN BINDING
AND MODE OF ADMINISTRATION ON
PENETRATION OF FIVE CEPHALOSPORINS
INTO SUBCUTANEOUS TISSUE FLUID IN HUMAN
BEINGS

B. Hoffstedt & M. Walder

The influence of serum protein binding and mode of administration on penetration of five cephalosporins into subcutaneous tissue fluid in human beings**

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** This study was partly presented at the 20th ICAAC in New Orleans, Louisiana, USA.

SUMMARY

The penetration of five cephalosporins into interstitial fluid was investigated by a new method employing cotton threads subcutaneously implanted. Penetration following short bolus injections, calculated as area under interstitial fluid level curve divided by area under serum level curve $\times 100$, was 47.0 % for cephradine, 30.6 % for cefuroxime, 26.7 % for cefotaxime, 24.6 % for cefoxitin and 9.6 % for cefoperazone. There was an inverse correlation between degree of penetration and serum protein binding with $r = -0.97$. The area under interstitial fluid level curves were the same whether the drugs were administered as short bolus injections or short time infusions.

It is generally maintainable that only the free, unbound, drug is available for antibacterial activity and for distribution to the extravascular compartments. Therefore, for treatment of infections outside the vascular compartment, drugs which are minimally bound to serum proteins are regarded as superior to drugs which are highly bound to the serum proteins (11, 14, 18). Although a number of models have been established to evaluate the influence of serum protein binding on antibiotic pharmacokinetics, especially in animals (4, 12, 17, 18, 20), all conclusions are far from certain (15).

It is likewise unclear as to what role the rates of drug delivery plays in penetration of the agent into the extravascular compartment. In one model an infusion for 15 minutes was preferential over an one hour infusion or a bolus injection because it yielded higher levels in subcutaneous cage fluid in rabbits (5). In another model the high peak was regarded as important for penetration into extravascular foci (3). In an effort to clarify these issues, we have investigated the penetration into the interstitial fluid (IF) of five cephalosporins in humans.

MATERIALS AND METHODS

The antimicrobials used were cephradine (serum protein binding capacity of 10 %) (13), cefuroxime (33 %) (7), cefotaxime (40 %) (6), cefoxitin (70 %) (8) and cefoperazone (90 %) (19). The serum protein binding capacities varied over the concentration range of the drugs but the figures mentioned are average values over the normal serum concentration range.

The investigation was approved by the Ethical Committee of the University of Lund and the volunteers were verbally informed and gave their verbal consent. Three or four healthy male and female volunteers with no known allergy to penicillin and no history of antimicrobial therapy during the last month were treated with each drug. Their mean age was 32.6 years $\pm$ 7.3 (SD) and the mean weight 70.4 kg $\pm$ 9.3 (SD). All had normal serum creatinines.

Each subject received one intravenous injection for five minutes and after at least one week an intravenous infusion for 30 minutes. The doses were 1 g of cephradine, 1.5 g of cefuroxime and 2 g of cefotaxime, cefoxitin and cefoperazone.

The model used for collecting subcutaneous interstitial fluid was that described by Ryan (16) and modified by Hoffstedt and Walder (9). After disinfection of the skin with an ethanol swab and instillation of a local anesthesia (1-2 ml Xylocain^R 10 mg/ml), two 22 cm long cotton threads (umbilical tape Ethicon^R) were inserted subcutaneously for 1-2 cm. The threads were then at the different sampling times drawn through the subcutaneous tissue and the positions previously placed subcutaneously cut off and immediately placed in a plastic tube with a volume of 300 μ l and then stored at -70°C until assayed.

Six to seven samples could be obtained from each thread.

Only thread samples without macroscopically visible blood were analysed.

Thirty minutes after the implantation of the threads (allowing the oedema caused by the anaesthesia to disappear) drugs were administered in the opposite arm. Samples from blood and threads were taken at 15, 60, 120, 240, 300, and 360 minutes after drug delivery.

Thread samples were analysed by a microbiological method. Disposable 24x24 cm plates containing 125 ml DST Oxoid CM 261 agar with pH adjusted to 7.4 were used with exception of cefotaxime where the agar was PDM-ASM-agar (Biodisk). Wells with a diameter of 8 mm were made by a regular hole punch machine. Then one cm of thread was cut into smaller pieces and spread out in the well. The antibiotic was eluted by adding 100 μ l of PBS-buffer to each thread containing well. Thread antibiotic standards were made by impregnating one cm of sterilized thread with different concentrations of antibiotic solutions.

Indicator organism for measurement of concentrations was for cephradine, cefuroxime, cefoxitin, and cefoperazone; Micrococcus luteus, ATCC 9341 and E. coli V6311/65 for cefotaxime. This indicator organism is susceptible to the desacetylmetabolite of cefotaxime. However, this metabolite has only 20 % of the antibacterial activity of the parent drug and as the degree of metabolism of cefotaxime was about 30 % the influence of this metabolite upon the obtained results was neglectable.

The limit of sensitivity for the assay methods was 0.5 μ g/ml for all drugs except cephradine and cefotaxime where the limit was 0.25 μ g/ml. The coefficient of variation (SD as percentage

of the mean concentration) varied for these assays from 8 to 13 %. This coefficient of variation was in the same range for the serum assays.

The penetration i.e. relation between area under the interstitial fluid curve (AUC_{IF}) and area under the serum curve (AUC_s) in per cent and relation between peak_{IF} level and peak_{serum} level in per cent for the tested drugs were calculated. The AUC was determined by the trapezoidal rule method. Half-life of the tested drugs in serum and IF and duration of IF levels above 2 $\mu\text{g/ml}$ were also calculated. The correlation between penetration and serum protein binding was determined from data on bolus injections and infusions.

Statistic methods. Statistical analyses were performed by using a paired t-test on differences on values of AUC_{IF} and AUC_s .

RESULTS

The data in Table 1 show that the mode of administration had only a small influence on the levels of the test drug in serum but had a marked influence on concentrations in IF. The IF peak levels were delayed when the drugs were administered as short time infusions. After one hour the concentration curves in IF after injection and infusion were almost parallel.

After a bolus injection the penetration was 47.0 % for cephadrine, 30.6 % for cefuroxime, 26.7 % for cefotaxime, 24.6 % for cefoxitin and 9.6 % for cefoperazone. Penetration after short time infusions were almost identical with exception of the most highly protein bound drugs, cefoxitin and cefoperazone which yielded somewhat higher figures. (Table 2).

The elimination half-lives of cephadrine, cefotaxime and cefoxitin were equal in serum and IF and no influence of mode

of administration could be seen. Cefuroxime and especially cefoperazone yielded a prolonged half-life in IF compared to serum. This prolonged half-life in IF also influenced the duration of IF levels above $2 \mu\text{g/ml}$. This time was about four hours for cefuroxime and five hours for cefoperazone compared to three hours for the other drugs (Table 2).

There was no statistic significant difference in the AUC_{IF} when the drugs were given as bolus injections compared to short time infusions.

The AUC_{S} varied between $299.9 \mu\text{g/ml} \times \text{h}$ (cefoperazone) to $36.5 \mu\text{g/ml} \times \text{h}$ (cephradine) and the AUC_{IF} between $40.8 \mu\text{g/ml} \times \text{h}$ (cefotaxime) to $18.6 \mu\text{g/ml} \times \text{h}$ (cefoxitin). (Table 2).

The relationships between peak levels in serum and IF varied between 41 % (cephradine) to 5 % (cefoperazone). (Table 2).

The correlation coefficient between degree of penetration measured as above mentioned and serum protein binding was $r = -0.97$. The correlation coefficient between $\frac{\text{AUC}_{\text{IF}}}{\text{dose}}$ and serum protein binding was $r = -0.67$.

DISCUSSION

In human beings Tan et al found a great influence of serum protein binding of penetration of antibiotics into extracellular fluid (18). Wise et al demonstrated a linked relationship between the degree of protein binding and the penetration of antibiotics (21).

In our study we have used a "physiological" model with a low degree of inflammatory reaction and tissue destruction. This was indicated by the low total protein content (9). We were able

to confirm the earlier results of Kunin et al. that only the free, unbound fraction of the antibiotic penetrates into the interstitial fluid (11). As the $\frac{AUC_{IF}}{dose}$ were almost identical for the low protein bound drugs and nearly twice that of the high protein bound cefoxitin and cefoperazone this indicates the necessity of administering high doses of highly serum protein bound drugs in order to achieve therapeutic levels in IF. Conversely low serum protein bound drugs might achieve high IF levels with small doses.

The absolute levels of antibiotics found in the interstitial fluid during our study are lower than those of Wise and Gillet (8). This can be explained by the fact that we have very little inflammatory reaction and tissue damage. Our levels and degree of penetration are also below those reported by Ryan who used a technique similar to ours (16). However, there is a major difference in that Ryan implanted the threads 24 hours before the antibiotic was administered and hence the volunteer developed an inflammatory reaction around the threads.

The shape of the interstitial fluid curves closely follow the serum curves indicating that only passive diffusion moves the drug to and from the extravascular compartment. Cefoperazone shows a prolonged half-life in interstitial fluid compared to serum. This may be due to its high protein binding capacity and resultant binding to IF proteins creating a "depot" like effect that can also be seen for other highly protein bound drugs as doxycycline (1, 10). The lipophilic effect of the long side chain in position 7 of cefoperazone could also be responsible for this "depot" like effect.

The influence of the mode of administration on the antibiotic levels in interstitial fluid is still under debate.

Carbon et al. found significantly higher IF fluid levels after 15 minutes infusion than after 1 hour infusion or bolus injection in rabbits (5). However, these values were achieved after two or three injections and a cumulative effect was noted in IF for all modes of administration. Auvergnat, on the other hand, found no differences in CSF levels of dogs of penicillin, ampicillin, and amoxycillin after several hours of therapy (2). Barza et al. found that in fibrin clots the levels of ampicillin were higher after intermittent administration than after constant infusion (3). The AUC_{IF} levels and the absolute levels in IF in our study are very similar to each other without any influence of mode of administration.

The levels in serum were sufficient for treating susceptible organisms with all tested drugs. In IF, however, the obtained levels were adequate for cephadrine, cefuroxime, cefotaxime and cefoxitin. Cefoperazone yielded levels in IF that were not sufficient for treating i.e. pseudomonas infections (Table 1).

Our conclusions from this study are that the penetration of an antibiotic into the subcutaneous interstitial fluid is inversely correlated to the serum protein binding of a drug when the penetration is measured as AUC_{IF} divided with AUC_S . As no differences in AUC_{IF} were shown due to mode of administration this might indicate that such factors as pain on injection, risk of thrombophlebitis or technical reasons might decide mode of administration.

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Table 1. Serum and interstitial fluid levels of the tested antibiotics.

Drug	Dose - g, and method of delivery	$\mu\text{g/ml}$ ($\pm$ SD)											
		Serum						Interstitial fluid					
		0.25 h	1 h	2 h	4 h	5 h	6 h	0.25 h	1 h	2 h	4 h	5 h	6 h
Cephadrine	1.0 s.b.	32.3 $\pm$ 10.6	11.5 $\pm$ 3.1	5.8 $\pm$ 1.8	1.8 $\pm$ 0.4	1.0 $\pm$ 0.3	0.5 $\pm$ 0.4	13.2 $\pm$ 5.7	9.4 $\pm$ 4.4	3.5 $\pm$ 1.3	1.7 $\pm$ 0.8	1.2 $\pm$ 0.6	0.7 $\pm$ 0.3
	1.0 inf.	21.5 $\pm$ 7.3	24.0 $\pm$ 8.1	5.1 $\pm$ 2.1	1.5 $\pm$ 0.3	0.8 $\pm$ 0.4	<0.25	1.5 $\pm$ 0.7	7.0 $\pm$ 3.2	2.1 $\pm$ 0.9	1.1 $\pm$ 0.5	0.6 $\pm$ 0.2	<0.25
Cefuroxime	1.5 s.b.	94.1 $\pm$ 8.2	45.1 $\pm$ 6.4	15.1 $\pm$ 3.4	3.6 $\pm$ 0.9	1.6 $\pm$ 0.3	0.7 $\pm$ 0.1	9.2 $\pm$ 3.7	11.8 $\pm$ 3.9	6.4 $\pm$ 2.1	2.0 $\pm$ 0.8	1.1 $\pm$ 0.6	0.7 $\pm$ 0.1
	1.5 inf.	91.3 $\pm$ 12.1	54.0 $\pm$ 8.9	20.3 $\pm$ 5.1	6.3 $\pm$ 1.6	2.9 $\pm$ 1.1	1.5 $\pm$ 0.6	2.1 $\pm$ 0.8	15.4 $\pm$ 4.1	7.0 $\pm$ 2.2	1.8 $\pm$ 0.6	1.1 $\pm$ 0.7	0.7 $\pm$ 0.1
Cefotaxime	2.0 s.b.	122.3 $\pm$ 11.1	50.1 $\pm$ 13.1	20.5 $\pm$ 4.9	4.7 $\pm$ 1.2	2.3 $\pm$ 0.6	1.0 $\pm$ 0.2	16.5 $\pm$ 2.4	12.7 $\pm$ 3.6	5.1 $\pm$ 2.8	0.8 $\pm$ 0.2	0.3 $\pm$ 0.2	<0.25
	2.0 inf.	109.2 $\pm$ 22.6	56.3 $\pm$ 12.7	15.9 $\pm$ 4.2	3.6 $\pm$ 0.9	1.8 $\pm$ 0.3	0.8 $\pm$ 0.1	7.1 $\pm$ 2.1	16.8 $\pm$ 0.3	7.4 $\pm$ 2.1	1.3 $\pm$ 0.4	0.3 $\pm$ 0.1	<0.25
Cefoxitin	2.0 s.b.	93.4 $\pm$ 9.7	31.2 $\pm$ 5.6	13.9 $\pm$ 3.1	1.9 $\pm$ 0.4	0.7 $\pm$ 0.2	<0.5	20.4 $\pm$ 3.7	17.1 $\pm$ 3.8	6.8 $\pm$ 1.5	0.9 $\pm$ 0.2	<0.5	<0.5
	2.0 inf.	76.4 $\pm$ 12.3	44.7 $\pm$ 8.7	12.4 $\pm$ 2.8	2.8 $\pm$ 0.9	1.3 $\pm$ 0.4	0.6 $\pm$ 0.1	14.2 $\pm$ 2.9	15.5 $\pm$ 2.9	5.0 $\pm$ 2.1	0.5 $\pm$ 0.1	<0.5	<0.5
Cefoperazone	2.0 s.b.	293.1 $\pm$ 42.4	104.1 $\pm$ 21.4	44.2 $\pm$ 7.9	21.0 $\pm$ 4.3	13.7 $\pm$ 2.9	8.6 $\pm$ 1.5	5.6 $\pm$ 1.1	8.0 $\pm$ 2.3	6.1 $\pm$ 1.4	3.4 $\pm$ 0.8	2.3 $\pm$ 0.6	1.7 $\pm$ 0.3
	2.0 inf.	182.3 $\pm$ 31.9	105.6 $\pm$ 29.1	49.9 $\pm$ 9.2	13.8 $\pm$ 4.1	7.8 $\pm$ 1.6	4.4 $\pm$ 0.9	1.2 $\pm$ 0.1	8.3 $\pm$ 1.9	6.2 $\pm$ 1.7	3.0 $\pm$ 1.0	2.1 $\pm$ 0.8	1.5 $\pm$ 0.4

Table 2. Doses and pharmacokinetic data of the tested cephalosporins.

Antibiotic	Serum Protein Binding %	Dose g	$t_s \pm \text{min}$	$t_{IF} \pm \text{min}$	Time in IF above $2 \mu\text{g/ml}$ h	$\text{AUC}_{IF} \mu\text{g/ml} \times \text{h}$	$\text{AUC}_S \mu\text{g/ml} \times \text{h}$	$\frac{\text{AUC}_{IF}}{\text{AUC}_S} \%$	$\frac{\text{Peak}_{IF}}{\text{Peak}_S} \%$
Cefradine	10	1.0 s.b.	61	55	3.5	20.4 ± 6.9	43.4 ± 14.7	47.0 ± 0.1	41
		1.0 inf.	54	50	3.0	18.8 ± 9.5	36.5 ± 7.0	51.5 ± 12.5	23
Cefuroxime	33	1.5 s.b.	52	70	4.0	36.3 ± 7.0	118.6 ± 22.8	30.6 ± 8.3	14
		1.5 inf.	51	82	3.7	25.7 ± 1.0	93.4 ± 19.7	27.5 ± 4.6	20
Cefotaxime	40	2.0 s.b.	50	45	3.0	40.8 ± 18.1	152.7 ± 20.4	26.7 ± 4.2	14
		2.0 inf.	50	45	3.5	39.8 ± 3.7	131.1 ± 24.1	30.4 ± 8.1	20.
Cefoxitin	70	2.0 s.b.	51	50	3.2	18.6 ± 7.6	75.6 ± 14.5	24.6 ± 4.5	22
		2.0 inf.	54	54	3.0	24.0 ± 17.3	56.9 ± 19.7	42.2 ± 10.9	26
Cefoperazone	90	2.0 s.b.	96	135	5.5	28.8 ± 3.0	299.9 ± 34.7	9.6 ± 1.3	5
		2.0 inf.	80	150	5.2	31.7 ± 3.7	215.1 ± 51.4	14.7 ± 6.1	6

COMPARISON OF SKIN BLISTERS AND
IMPLANTED COTTON THREADS TO EVALUATE
ANTIBIOTIC TISSUE CONCENTRATIONS

B. Hoffstedt, M. Walder & A. Forsgren

Antibiotic concentration in skin blisters and cotton threads

Comparison of Skin Blisters and Implanted Cotton Threads to
Evaluate Antibiotic Tissue Concentrations

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The purpose of this study was to compare a skin blister technique and a new technique with subcutaneously implanted cotton threads to evaluate antibiotic tissue concentrations. Six volunteers received 2 g ampicillin and, after at least one week, 1 g of cloxacillin intravenously. Samples from serum, blister fluid and thread fluid were analysed by an agar well diffusion technique. The half-life for ampicillin and cloxacillin was prolonged in blister fluid compared to serum and thread fluid. Our conclusions are that the thread technique reflects the kinetics of antibiotics in extracellular fluid where the penetration into it mostly depends on the serum protein binding of the drug. The blister technique, on the other hand resembles the condition in different subcutaneously implanted cages, fibrin clots and even abscesses where such factors as time and volume of the extracellular compartments have great influence on the distribution of the antibiotics.

Introduction

Several methods have been used to evaluate the distribution of antimicrobial agents in extracellular fluid in animals (1, 2, 3). However, studies on the penetration of these substances into human extracellular fluid are rare. The most commonly used methods in humans are the skin windows (4), skin blisters induced by cantharidine (5) and skin blisters induced by suction (6). A new method to obtain extracellular fluid using subcutaneously implanted cotton threads has recently been described (7, 8).

The purpose of the present study was to compare this new method with an established one (6). As reference method the suction blister technique was chosen due to our earlier experience developing blisters by suction for other purposes (9) and because many recently published papers refer to that method.

Materials and methods

Healthy Volunteers

The study comprised six male volunteers aged from 21 to 38 years. Their mean body weight was 73.5 ± 11.3 (SD) kg. The investigation was approved by the Ethical Committee of the University of Lund and the human volunteers were verbally informed of the conceivable risks and gave their verbal consent. The volunteers were healthy with no concurrent disease or known allergy to penicillin, and had not been on antimicrobial therapy during the last two weeks.

Antibiotic and Dosage

Two different antibiotics were studied; ampicillin with low serum protein binding, and cloxacillin, with high serum protein binding. Single doses of 1 g and 2 g respectively were administered intravenously as five minutes bolus injections. All volunteers received both antibiotics with an interval of at least one week between antibiotics.

Experimental Procedure

After disinfection of the skin with an ethanol swab, skin blisters were produced by tightly strapping a perspex block with eight bores (diameter 8 mm and 10 mm apart) to the volar aspect of one forearm. Using a suction pump (model junior DS, SCRICAR, Zurich, Switzerland) a negative pressure of 0.4 kg/cm^2 was applied for one hour and a negative pressure of 0.6 kg/cm^2 for an additional half hour. After that time the blisters were fully developed, containing 50 to 150 μl fluid each. The blisters were punctured with a thin sterile injection canule and the fluid was collected in a microcapillar tube.

Below this perspex block on the lateral side of the forearm two cotton threads were subcutaneously implanted as described previously (7). In brief, 1-2 cm of a 20 cm long thread was inserted subcutaneously after disinfection of the skin and local anesthesia (2-3 ml Lidocain 10 mg/ml). The piece of thread being subcutaneously was pulled out and cut off and placed in a plastic tube with a volume of 300 μ l. At the same time a new piece of the same thread was drawn in subcutaneously to permit another sample.

The threads were implanted for the first time at least 30 minutes and the blisters were fully developed at least 15 minutes before the antibiotic was delivered.

Sampling Schedule

Samples were taken from blood, blisters and threads 15, 45, 90, 150, 240 and 300 minutes after the infusion was started. The arm used for the sampling was never the arm used for injection of antibiotics.

Samples were then stored at -70°C until assay which was within seven days.

Antibiotic Assay

The total concentrations of the antibiotics in serum, blister fluid and thread fluid were analysed by a microbiological method. For details on assay of cotton threads see Hoffstedt & Walder (7). Disposable 24x24 cm plates containing 125 ml DST Oxoid agar with pH adjusted to 7.4 were used. The indicator organism was Micrococcus luteus ATCC 9341. Wells with a diameter of 8 mm were made by a regular hole punch machine. No antibacterial effect was seen for blister/thread fluid when no antibiotic was administered.

Standard antibiotic concentrations for assay of serum were made by diluting an antibiotic solution with serum and for assay of skin blister fluid by diluting with a solution of blister fluid and saline in the proportion 1:5. Previous experiments had yielded the same results with this mixture as with pure skin blister fluid. All blister samples and standards were diluted 1:5 then stored at -70°C for the same period as the test samples. All blisters, serum and thread samples were assayed in duplicate. The range of the assay for the thread fluid containing ampicillin and cloxacillin was 0.5-50 $\mu\text{g/ml}$.

The coefficient of variation (SD as percentage of the mean concentration) varied for the thread fluid assay from 8 to 13 % in different experiments.

Antibiotic and Albumin Binding of Threads

Twelve threads were impregnated with unlabeled ampicillin and 12 threads with ^{35}S -ampicillin-trihydrat (specific activity 4.9 $\mu\text{Ci/mg}$) in a concentration of 100 $\mu\text{g/ml}$. Twelve threads were impregnated with a 100 $\mu\text{g/ml}$ solution of unlabeled cloxacillin and 12 threads with ^{35}S -cloxacillin-Na (specific activity 4.4 $\mu\text{Ci/mg}$). Finally 12 threads were impregnated with a solution of ^{125}I labeled albumin of the concentration 20 g/l, corresponding that of interstitial fluid.

The impregnated threads were eluted in separate baths containing 1000 ml saline during stirring for 30, 60, 90, 120 minutes and 16 hours. The threads were moved to a fresh bath after 120 minutes. Remaining antibiotics and albumin were measured with antibiotic assay and automatic gamma counter respectively and compared with threads that had been impregnated but not eluted. Two threads were used for each sampling time.

iodine per mole protein. Albumin labeling was performed by Dr. Bengt-Göran Hansson, the Department of Clinical Virology, the General Hospital, Malmö. Radiolabeled ampicillin and cloxacillin were obtained from ASTRA, Sweden. The radioactivity in the threads was measured in an automatic gamma counter (LKB-Wallac 80,000) and in a liquid scintillation counter (Packard, Model B2450).

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Calculations

From the mean ($n = 6$) serum, blister fluid and thread fluid levels the following parameters were calculated: half-life, area under the curve (AUC) (by the trapezoidal method), penetrability ($\frac{AUC_{ECF}}{AUC_S} \times 100 \%$), i.e. area under extracellular fluid (ECF) level curve divided by the area under serum (S) level curve multiplied by 100. The relationships between the peak levels in serum and extracellular fluid were also calculated.

Protein Determination

The concentrations of protein in blister fluid and thread fluid were measured by a modified Mancini-technique by Dr. A. Grubb, Department of Clinical Chemistry, Malmö General Hospital, Malmö.

Radiolabeling Procedure and Measurement

The radiolabeling of human albumin preparations was done in the following way: Human albumin was radioiodinated (^{125}I , The Radiochemical Centre, Amersham) to high specific activity with lactoperoxidase coupled to polyacrylamide exactly as described by Thorell & Larsson (10). The preparations contained approximately 0.05 mole iodine per mole protein. Albumin labeling was performed by Dr. Bengt-Göran Hansson, the Department of Clinical Virology, the General Hospital, Malmö. Radiolabeled ampicillin and cloxacillin were obtained from ASTRA, Sweden. The radioactivity in the threads was measured in an automatic gamma counter (LKB-Wallac 80,000) and in a liquid scintillation counter (Packard, Model B2450).

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Results

Figure 1 demonstrates serum, blister fluid and thread fluid levels after infusion of 1 g cloxacillin. As can be seen from the figure the entry of cloxacillin into both thread fluid and blister fluid is delayed, with a maximum peak level after 45-90 minutes. In contrast, Figure 2 demonstrates fast entry of ampicillin in both extracellular fluids.

As can be seen from Table 1 and Figures 1 and 2, the half-life of both ampicillin and cloxacillin in blister fluid is prolonged, compared to the half-life in serum and thread fluid. However, half-lives of both antibiotics in thread fluid are approximately the same as in serum. The relationships between peak levels in serum and threads or blister fluid are presented in Table 1. Notable is the very low ratio of cloxacillin.

As can be seen from Table 2 the AUC of the blister fluid is about $2\frac{1}{2}$ times that of the AUC of the thread fluid for ampicillin. The AUC_S of ampicillin is three times that of cloxacillin. The AUC of the thread fluid for ampicillin is almost eight times that of cloxacillin.

The penetrability into the thread fluid, i.e. $AUC \text{ thread fluid} / AUC \text{ serum}$, is also demonstrated in Table 2. For ampicillin penetrability of 26 % and for cloxacillin of 12 % was calculated. The penetrability of ampicillin into blister fluid ($AUC \text{ blister fluid} / AUC \text{ serum}$) was estimated to be 68 %. The half-life for cloxacillin in the blister fluid is so prolonged that no calculation of penetrability can be performed.

The protein concentrations in serum, blister fluid and thread fluid are presented in Table 3. The albumin level in the thread fluid is slightly lower than in the blister fluid.

To check if ampicillin, cloxacillin or albumin were bound to the cotton threads, threads were impregnated with the antibiotics or radiolabeled albumin and subsequently eluted in saline.

For the threads that had been impregnated with ampicillin or cloxacillin in a concentration of 100 µg/ml and then eluted in saline for 30 minutes, less than 0.5 µg/ml could be detected when measured with bacteriological or radiological method.

The radioactivity left in the threads impregnated with ^{125}I labeled albumin after elution in a saline bath was on the average 20 % after 30 minutes elution. No further decline was seen.

Discussion

Ever since Chisholm et al. (2) presented their technique using subcutaneously implanted cages for the study of antibiotic distribution into extracellular fluid, there has been a debate on the relevance of these cages for measuring penetration of antimicrobial agents from blood into extracellular fluid (11). The slow penetration of the antibiotics into implanted cages and especially the prolonged elimination of the drugs from them raised doubts whether the chamber fluid was really equivalent to interstitial fluid. As has been shown by Ryan & Mason, the antibiotic levels in tissue cage fluid differ significantly from the levels in subcutaneous fluid (11). Furthermore, they could show that the antibiotic in the tissue cage fluid is not in the same degree of dynamic equilibrium with blood, as is the antibiotic in normal subcutaneous fluid. These differences were attributed to the relatively impermeable granulomatous layer surrounding tissue cages (11).

A similar delay in elimination of the antibiotics was also evident in the method using subcutaneously implanted fibrin clots (1). The kinetics of antibiotics in clots closely resembled abscesses. On the other hand, the same authors found that antibiotics in interstitial fluid were eliminated faster and the elimination was more similar to that in serum (1).

Skin blister fluid has shown the same phenomenon of prolonged elimination of the drugs (5, 6, 12). The pharmacokinetics of antibiotics in blister fluid are similar to that of different subcutaneous tissue cages and implanted fibrin clots (1, 2).

Another aspect of the blister technique is stressed by the results obtained by protein determination in the blister fluid. A higher concentration than in true extracellular fluid has been demonstrated, indicating a leakage from serum (6). In our study the albumin concentration in the blister fluid was less than what Schreiner et al. have found and similar to interstitial fluid.

The low remaining antibiotic concentrations in the threads after elution demonstrated in this study indicate that no pronounced binding of the tested drugs to the used cotton threads existed. However, the amount of albumin bound to the threads was 20 %. This binding might influence the results in the reduction of the cloxacillin thread fluid concentrations from 45 to 90 minutes as it is somewhat slow. However, the influence of this binding cannot be of major importance since the elimination half-life of cloxacillin in the thread fluid is the same as in serum. If this influence would be pronounced a depot effect should be obvious especially for highly protein bound drugs.

The thread fluid concentration curves of both cloxacillin and ampicillin almost paralleled that of the serum, indicating a fast dynamic equilibrium between antibiotics in the blood and the thread fluid. Hence the thread fluid levels might closely reflect the situation in true interstitial fluid. This interpretation was further supported by the analysis of the thread fluid (7).

The antibiotic levels in the blister fluid, on the other hand, were characterized by a prolonged elimination time, blister fluid levels exceeding serum levels after a couple of hours. This delayed

elimination could be explained by a large blister volume compared to the surface through which the drug penetrates.

These differences in antibiotic pharmacokinetics of the blister and the thread fluid were more pronounced for cloxacillin, a drug with a high degree of serum protein binding. The peak blister fluid levels of cloxacillin were much lower than those in the thread fluid. Even the peak level time was delayed and the elimination from the blister fluid was prolonged. This indicates the existence of a factor limiting e.g. protein binding, the entry and elimination of cloxacillin. One reason for the low blister fluid levels of cloxacillin may be the time required for establishment of equilibrium between serum and blister concentrations. The time taken for the drug to penetrate into the large blister fluid compartment before the serum level declines is too short.

Our conclusions from this comparative study are that the thread technique reflects the kinetics of antimicrobials in extracellular fluid of subcutaneous tissue and hence a "shallow compartment" and that the degree of penetration into the extracellular fluid significantly depends on the serum protein binding of the drug. On the other hand, the blister technique with blisters made prior to the administration of the drugs resembles the condition in different subcutaneously implanted cages, fibrin clots, and hence a deep compartment, where such factors as time and volume of the extracellular compartments have a great impact on the apparent distribution of the antibiotics.

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Table 1: Pharmacokinetic data of ampicillin and cloxacillin ($t_{1/2}$ = half-life). Mean of six volunteers.

Antibiotic	$t_{1/2}$ serum min	$t_{1/2}$ thread min	$t_{1/2}$ blister min	$\frac{\text{Peak}_{\text{thread}}}{\text{Peak}_{\text{serum}}} \times 100\%$	$\frac{\text{Peak}_{\text{blister}}}{\text{Peak}_{\text{serum}}} \times 100\%$
Ampicillin	50	50	105	23	32
Cloxacillin	39	36	Not possible to calculate	5	2

Table 2: Pharmacokinetic data of ampicillin and cloxacillin. Mean of six volunteers.

Antibiotic	AUC_{serum} $\mu\text{g/ml} \times \text{h}$	AUC_{thread} $\mu\text{g/ml} \times \text{h}$	AUC_{blister} $\mu\text{g/ml} \times \text{h}$	$\frac{AUC_{\text{thread}}}{AUC_{\text{serum}}} \times 100\%$	$\frac{AUC_{\text{blister}}}{AUC_{\text{serum}}} \times 100\%$
Ampicillin	145	38	99	26	68
Cloxacillin	44	5	Not possible to calculate	12	Not possible to calculate

Table 3: Protein content in blister fluid, thread fluid and serum.
Mean values of six volunteers.

	Blister fluid	Thread fluid	Serum
Albumin (g/l)	19.3 $\pm$ 3.9	16.0 $\pm$ 1.0	45.3 $\pm$ 2.5
IgG (g/l)	4.2 $\pm$ 1.3	Not done	11.0 $\pm$ 0.9

LEGENDS TO FIGURES

Figure 1: Serum (——), blister fluid (— · —), and thread fluid (— — —) levels (mean values of six volunteers) after infusion of 1 g cloxacillin i.v. Vertical bars represent standard deviation.

Figure 2: Serum (——), blister fluid (— · —), and thread fluid (— — —) levels (mean values of six volunteers) after infusion of 2 g ampicillin i.v. Vertical bars represent standard deviation.

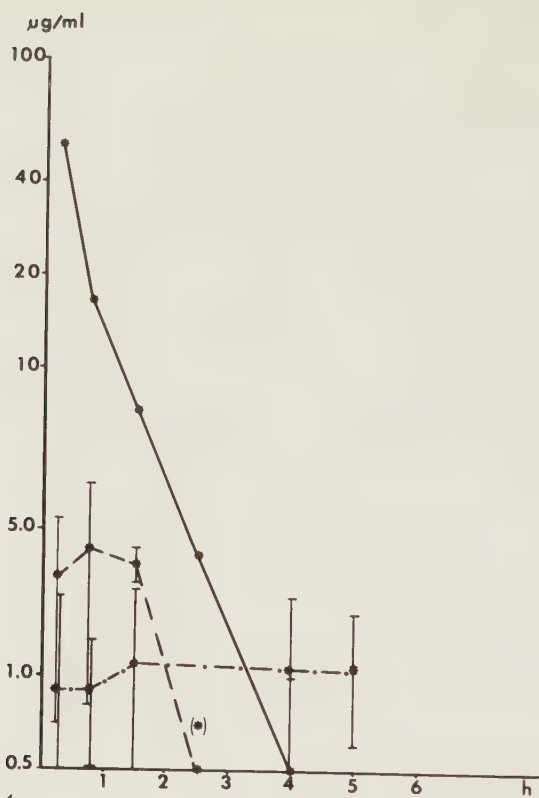


Fig. 1

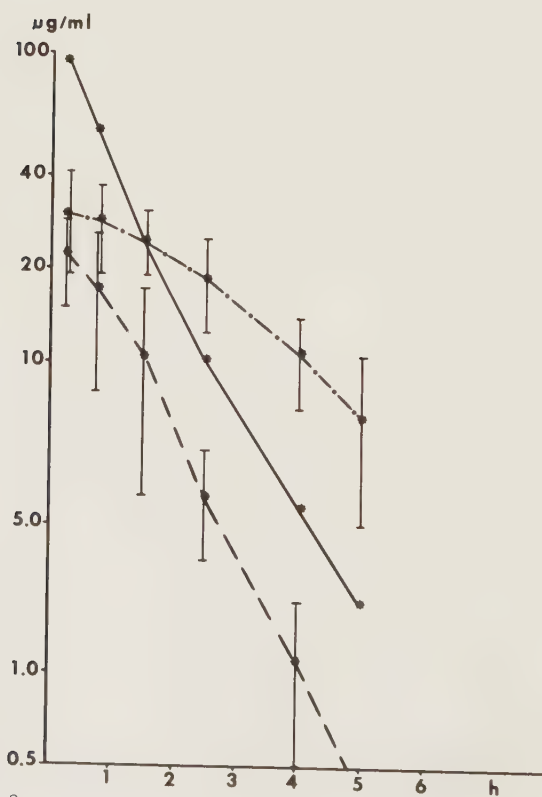


Fig. 2

PENETRATION OF CEFTAZIDIME INTO
EXTRACELLULAR FLUID IN PATIENTS

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Penetration of ceftazidime into extracellular fluid in patients

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Eight patients were treated with ceftazidime, four of whom had impaired renal function with creatinine clearances below 60 ml/min per 1.73 m². The levels of ceftazidime in serum and extracellular fluid were determined. Half-life in serum and extracellular fluid was 2.5 and 2.2 h respectively for patients with normal renal function. For patients with impaired renal function the half-life of the drug was 7.9 and 11.5 h, respectively.

The penetration of ceftazidime into extracellular fluid was around 50% in both groups. The levels achieved in both serum and extracellular fluid were satisfactorily high and prolonged, suggesting that a dosage regimen of 1 g twice daily is satisfactory.

Introduction

One of the most important features for therapeutic success is that the administered antibiotic achieves appropriate concentration at the site of infection. Since many infections start and spread in the extracellular fluid (ECF) the antibiotic levels obtained in this fluid is of importance to the clinical outcome. Ryan (1979) presented a convenient method of collecting ECF. A modified method has been used in animals, human volunteers and in patients without problem (Hoffstedt & Walder, 1981). In this study we have used this method for measuring levels of ceftazidime, in serum and ECF.

Materials and methods

The study was approved by the Ethical Committee of the University of Lund and the patients were verbally informed and gave their consent.

Eight patients, age range 46-72 years, average weight 73.3 ± 13.8 kg, were treated with ceftazidime. The renal function was checked on the day of admission and on the day of testing by measuring serum creatinine. Creatinine clearance was calculated by the method of Siersbaek-Nielsen *et al.* (1971). The patients were divided into two groups according to their creatinine clearance. Group A with clearance ≥ 60 ml/min $\times$ 1.73 m² and group B with clearance < 60 ml/min $\times$ 1.73 m². There were four patients with a mean creatinine clearance in group A of 76 ml/min $\times$ 1.73 m² and in group B of 45 ml/min $\times$ 1.73 m².

A dosage of ceftazidime 1 g three times daily was administered as short bolus infusions over 5 min. One patient, however, received 1 g twice daily because of severely impaired renal function.

On the lateral side of one forearm of each patient, two cotton threads were subcutaneously implanted after disinfection of the skin and applying 2-3 ml local anaesthesia. The thread samples were then gradually drawn through subcutaneously and then cut. The cut off piece of thread was then immediately placed in a small plastic tube. The blood samples were obtained by venepuncture.

Samples from blood and threads were taken after at least 3 days of therapy when a steady state could be expected. Blood and thread samples were taken 15, 45, 120, 240 and 300 min after the drug was administered.

The samples from blood and threads were stored at -70°C until assayed the following day. The serum samples were assayed by an ordinary agar well diffusion technique using a spore suspension of *Bacillus subtilis* 1904E as the indicator organism.

The thread samples were also assayed by an agar well diffusion technique. One cm of thread was cut in pieces and spread out in an 8 mm well in an agar plate. One hundred μl of buffer was added to each well to ensure an even distribution of the antibiotic. The agar used was DST in 12 in. $\times$ 12 in. disposable plastic assay plates and the indicator organism was a strain of *Proteus vulgaris*. Thread-antibiotic standards were made by impregnating 1 cm of sterilized thread with different concentrations of antibiotic solutions. Both serum and thread samples were assayed in duplicate.

From the mean values of serum and ECF levels, the half-life of ceftazidime was calculated. From the same parameters, the area under the extracellular fluid level curve (AUC_{ECF}) and area under the serum level curve (AUC_s) were determined by the trapezoidal rule method. The relationship between peak levels in serum and ECF and between AUC_{ECF} and AUC_s were also calculated.

Results

The half-life of ceftazidime in ECF and serum was 2.2 and 2.5 h, respectively, for group A, and for group B was 11.5 and 7.9 h, respectively. The relationships between peak levels in ECF and serum and between AUC_{ECF} and AUC_s was approximately 50% for both groups. However the values for AUC_{ECF} and AUC_s for group B were almost twice those obtained by group A (Table I).

As can be seen from Figure 1 the initial ECF and serum levels were about the same in both groups although the levels declined much more slowly in group B.

Discussion

The half-life in serum of ceftazidime was longer in these patients than that reported in normal subjects (O'Callaghan *et al.*, 1980). Even in group A the serum half-life of the drug was prolonged compared to human volunteers. This prolonged half-life combined with high ECF and serum levels, up to 5 h after injection of the drug, indicated that a dose regimen of two doses a day would be sufficient in treating most patients.

The penetration was equal or superior to other cephalosporins (Hoffstedt & Walder, 1981). The achieved levels of ceftazidime in both ECF and serum were well above the MIC for the pathogens susceptible to the drug. This indicated that a dose of 1 g was adequate. The high initial levels in ECF of about 27 mg/l suggested that ceftazidime may be effective in the treatment of infections not only in blood and urine but also in soft tissues.

Table I. Pharmacokinetic data of ceftazidime. Mean values ($n=4$)

Group	Creatinine clearance $\text{ml/min} \times 1.73 \text{ m}^2$	Half-life in serum (h)	Half-life in ECF (h)	$\frac{\text{Peak}_{\text{ECF}}}{\text{Peak}_{\text{serum}}} \times 100\%$	$\frac{\text{AUC}_{\text{ECF}}}{\text{mg/l} \times \text{h}}$	$\frac{\text{AUC}_{\text{serum}}}{\text{mg l} \times \text{h}}$	$\frac{\text{AUC}_{\text{ECF}}}{\text{AUC}_{\text{serum}}} \times 100\%$
A	76	2.5	2.2	46	95	184	52
B	45	7.9	11.5	44	163	282	58

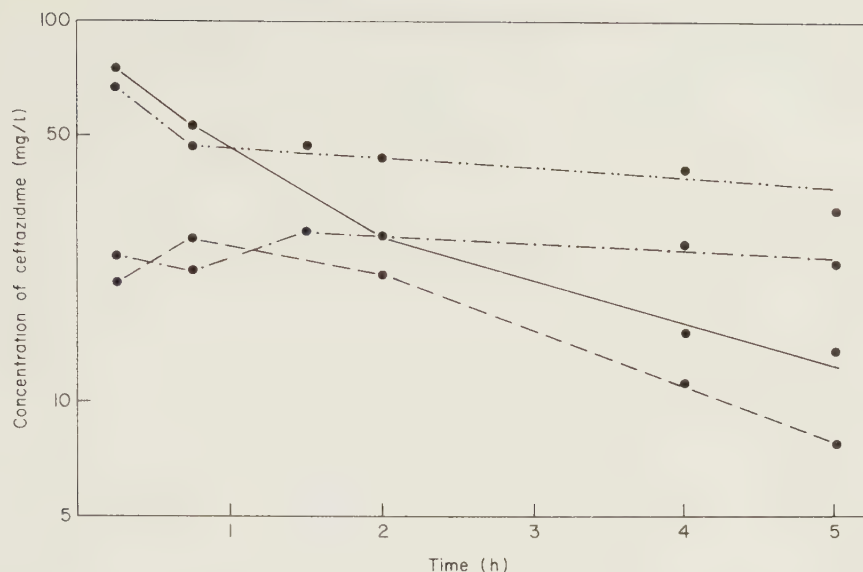


Figure 1. Serum and extracellular fluid levels of ceftazidime in patients, —, mean serum level in patients with creatinine clearance ≥ 60 ml/min $\times 1.73$ m²; ---, mean extracellular fluid level in patients with creatinine clearance ≥ 60 ml/min $\times 1.73$ m²; - · - · -, mean serum level in patients with creatinine clearance < 60 ml/min $\times 1.73$ m²; — — —, mean extracellular fluid level in patients with creatinine clearance < 60 ml/min $\times 1.73$ m².

Our conclusions from the study are that ceftazidime has very favourable pharmacokinetic properties with a long half-life both in serum and ECF, good penetrability into ECF and satisfactorily high levels both in serum and ECF after a 1 g dose. It is likely that a dose regime of 1 g twice daily will be sufficient for most patients.

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PENETRATION OF FOSFOMYCIN AND
GENTAMICIN INTO THE INTERSTITIAL
FLUID

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Penetration of fosfomycin and gentamicin into the interstitial
fluid**

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**The study was partly presented at the 12th ICC in Florence,
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Summary

To establish the influence of moderate difference in molecular size of antimicrobials on penetration into the interstitial fluid, 1 g fosfomycin (MW = 138 D) and 120 mg gentamicin (MW = 530 D) was administered intravenously to human volunteers. Samples of interstitial fluid were obtained by subcutaneously implanted cotton threads. Serum and thread samples were analysed microbiologically with an agar Well diffusion technique.

As penetration of fosfomycin (serum protein binding 0 %) was 36.6 ± 11.7 % and of gentamicin (serum protein binding 0 %) 66.8 ± 13.2 % no positive correlation could be demonstrated between molecular size and penetration into interstitial fluid. Hence other physical properties of the drugs might influence the penetration.

Fosfomycin yielded the same elimination half-life, 2 hrs in serum as in interstitial fluid but the half-life of gentamicin in interstitial fluid was prolonged, 2.5 hrs compared to 2 hrs in serum.

The obtained levels of gentamicin in interstitial fluid were comparatively low with peak level of $2.4 \mu\text{g/ml}$.

The distribution of antimicrobial agents from the blood stream through the capillary pores into the surrounding interstitial fluid is said to take place as a free diffusion of the non serum protein bound part of the drug (5). These pores permit the passage of substances with a molecular weight up to the size of small plasma proteins (4).

In order to investigate if there was any difference in penetration of antibiotics into the interstitial fluid depending on the molecular size we studied penetration of fosfomycin and gentamicin, two agents with differences in molecular weight. Either drug have no serum protein binding capacity at normal therapeutic serum levels.

MATERIAL AND METHODS

Healthy volunteers. The study comprised of six healthy female and male volunteers aged 23-41 years. Their mean body weight was 62.5 ± 12.4 (SD) kg. The investigation was aproved by the Ethical Committee of the University of Lund and the human volunteers were verbally informed and gave their verbal consent. The volunteers were healthy with no concurrent disease and had no known allergy to fosfomycin or gentamicin. They had no history of antimicrobial therapy during the last two weeks.

Antibiotic and dosage. Two different antibiotics were studied; one with low molecular weight = 138 D, fosfomycin (by courtesy of Dumex Ltd, Copenhagen, Denmark) and one with moderately high molecular weight = 530 D, gentamicin (Garamycina^R, Essex, Sweden). The doses given were 1 g of fosfomycin and 120 mg of gentamicin as short bolus injections intravenously for 5 minutes.

Experimental procedure. For further details see Hoffstedt and Walden

In brief, two pieces of a cotton thread were inserted for 1-2 cm subcutaneously after disinfection of the skin. The threads were then gradually drawn through the subcutaneous tissue and positions that had been placed subcutaneously were cut off and then immediately placed in a plastic tube with a volume of 300 μ l and frozen at -70°C until assayed.

Sampling schedule. Samples from blood and threads were taken at 15, 45, 90, 150, 240, 270, and 360 minutes after the start of fosfomycin-injection and at 15, 60, 120, 240, and 300 minutes after the start of gentamicin-injection. The arm used for the sampling was always opposite to the arm used for the injection of antibiotics.

Antibiotic assay. The total concentrations of the antibiotics in serum and thread fluid were analysed microbiologically using anagar Well diffusion technique. Disposable 24x24 cm plates containing 125 ml DST Oxoid agar with pH adjusted to 7.4 were used. The indicator organism was for gentamicin *Staphylococcus epidermidis* 5212 and for fosfomycin *Proteus vulgaris* ATCC21100. Wells with a diameter of 8 mm were made by a regular hole punch machine. All thread and serum samples were assayed in duplicate.

Calculations. From the obtained levels in serum and thread fluid the following parameters were calculated, half-life, area under the curve (AUC) (by the trapezoidal method), penetration $= \left(\frac{\text{AUC}_{\text{IF}}}{\text{AUC}_{\text{S}}} \times 100 \% \right)$, that is area under interstitial fluid level curve divided by area under serum level curve multiplied with 100. The relationship between the peak levels in serum and interstitial fluid was also calculated.

RESULTS

Figure 1 demonstrates serum and interstitial fluid levels after injection of 1 g fosfomycin. There is a fast entry into the interstitial fluid with a peak level after 15 minutes. Then the concentrations decline and run parallel to the serum concentrations.

Figure 2 demonstrates the corresponding curves for gentamicin. The peak level in interstitial fluid is delayed compared with the serum peak level and the elimination half-life in the interstitial fluid is somewhat prolonged and hence not parallel to the serum curve.

The elimination half-life of fosfomycin is two hours in both serum and interstitial fluid (Table 1). That is in contrast to gentamicin that have a prolonged half-life in interstitial fluid. Notable is also the gentamicin peak level of $2.4 \mu\text{g/ml}$ in the interstitial fluid.

From Table 2 it can be seen that the AUC_s of fosfomycin is about 11 times that of gentamicin and that AUC_{IF} of fosfomycin is about 7 times that of gentamicin. However, the relations between $\frac{\text{peak}_{\text{IF}}}{\text{peak}_s}$ and $\frac{\text{AUC}_{\text{IF}}}{\text{AUC}_s}$ are for fosfomycin about half of the corresponding relations of gentamicin.

DISCUSSION

The spatium that allows a lipid insoluble molecule to cross a typical capillary endothel is of the size that even plasma protein molecules can pass (4, 6). Chisholm et al. (2) suggested that the distribution of an antimicrobial agent into a subcutaneous tissue cage was restrained by the degree of protein binding and that the

molecular weight of the tested drugs were of neglectable importance.

In this study with two drugs with no protein binding no positive correlation between molecular size and penetration into the interstitial fluid was found. On the contrary, the largest molecule (gentamicin) yielded the best penetration. As both fosfomycin and gentamicin have a neglectable binding to serum proteins other physical factors might influence this penetration.

The concentrations of fosfomycin in interstitial fluid were similar to the results obtained by Vicente et al. (7) who investigated the penetration of fosfomycin into a skin window fluid in dogs. They found that the peak levels in interstitial fluid were somewhat delayed compared to serum but the elimination half-life in interstitial fluid was the same.

The prolonged elimination half-life of gentamicin in the interstitial fluid compared to the serum half-life might depend on a binding of gentamicin to substances in the interstitial fluid or interstitial space. This "depot" effect can of course also partly be responsible for the better penetration of gentamicin compared to fosfomycin that we found. A prolonged half-life was also demonstrated for gentamicin by Chisholm et al. (1) but in that case it mostly depended on the used technique with implanted tissue cages.

The interstitial fluid levels of gentamicin obtained in this study with a maximum value of $2.4 \mu\text{g/ml}$ were low in relation to the MIC for most susceptible bacterias. On the other hand the serum levels well exceeded the MICs. These results may explain the negative clinical experience of gentamicin treatment of infections outside the vascular and urinary compartments. Due to risk of toxic side effects it is also problematic to increase the interstitial fluid levels of gentamicin by administering

large doses. This is in contrast to the non toxic drug fosfomycin where the levels easily can be raised by increasing the delivered dose.

The conclusions from this study are that firstly the moderate difference in molecular size between fosfomycin and gentamicin does not influence the penetration into interstitial fluid and secondly the obtained low levels of gentamicin in interstitial fluid might explain the problems of treating infections in tissue compartments with this drug.

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TAB. 1 - Elimination half-lives and peak levels of fosfomycin and gentamicin in serum and interstitial fluid

Drug	Elimination half-life serum (hrs)	Elimination half-life thread fluid (hrs)	Peak level $\mu\text{g/ml}$ serum	Peak level thread fluid $\mu\text{g/ml}$
Fosfomycin	2	2	94.0 ± 38.0	19.0 ± 6.0
Gentamicin	1.5	2.5	7.0 ± 1.0	2.4 ± 0.3

TAB. 2 - Area under the curves for fosfomycin and gentamicin in serum and insterstitial fluid and the relationship between them. Relationship between peak levels in serum and insterstitial fluid

Drug	Area under serum level curve $\mu\text{g/ml} \times \text{h}$	Area under thread fluid level curve $\mu\text{g/ml} \times \text{h}$	Peak thread fluid Peak serum	$\frac{\text{AUC}_{\text{IF}}}{\text{AUC}_S} \%$
Fosfomycin	160.0 ± 22.5	59.2 ± 20.4	23.4 ± 10.6	36.8 ± 11.7
Gentamicin	13.8 ± 2.2	9.3 ± 0.2	43.8 ± 11.6	66.8 ± 13.2

LEGENDS TO FIGURES

Figure 1 - Serum and interstitial fluid levels after injection of 1 g fosfomycin intravenously. Mean values of three volunteers.

Continuous line: Serum levels

Broken line: Interstitial fluid levels

Vertical bars: Standard deviation

Figure 2 - Serum and interstitial fluid levels after injection of 120 mg gentamicin intravenously. Mean values of three volunteers.

Continuous line: Serum levels

Broken line: Interstitial fluid levels

Vertical bars: Standard deviation

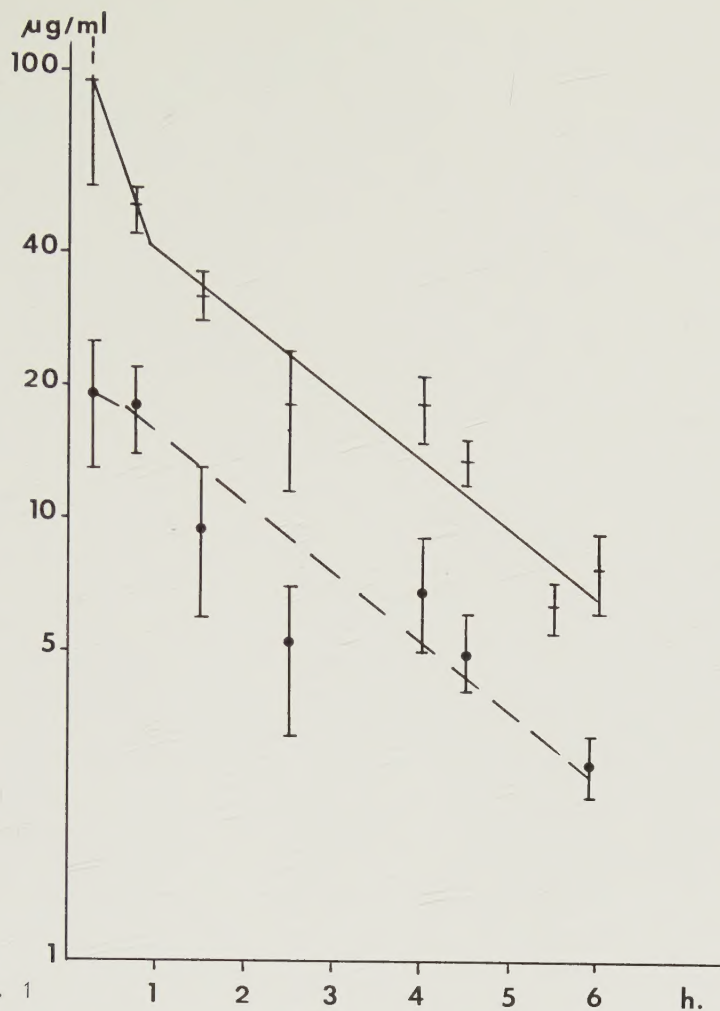


Fig. 1

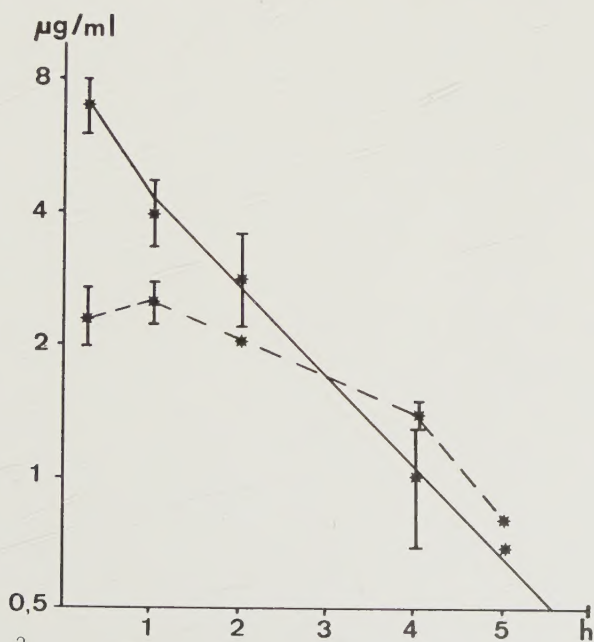


Fig. 2

